using supercoiled DNA. Seidel *et al.*[9] found that negative supercoiling slowed translocation by roughly an order of magnitude but eventually all negative supercoils were removed and further positive supercoils were created in front of the translocating enzyme. This topological change is achieved without separating the strands of DNA, so although the force required to stall the enzyme is not particularly large when compared to other motors moving on DNA[10], the enzyme can generate significant torque.

One question has vexed those interested in the type I restriction enzymes: how does translocation commence close to the target specificity sequence? It is not conceptually difficult to imagine that once the motor has translocated a substantial amount of DNA, it should be easy to continue this process[17]. However, the initial formation of the small translocated loop of DNA when it is only a few or tens of base pairs in length will require major structural distortion of the DNA and a lot of energy from ATP hydrolysis[17]. This would suggest that the initiation of translocation is a rare event with many abortive cycles being attempted by the motor before it finally grabs strongly enough to the DNA to hold the loop and to commence enlargement of the loop. The tweezers experiments of Seidel *et al.*[9] only measured translocation and are not sensitive to this initiation period. Nevertheless, the authors report that they are conducting further experiments that vary the concentration of the enzyme; such experiments may be able to address this question.

The few researchers who have over many years championed the type I restriction enzymes as extraordinary molecular machines find the great complexity of the molecules fascinating. Modern biophysical techniques are being applied to increasingly complex biological machines and it is very pleasing to see that it is now, at last, the turn of the type I restriction enzymes for the dynamic single-molecule treatment. Further single-molecule experiments complemented by traditional methods are expected to reveal much about the mechanism of these machines. The initiation of translocation, the nature of the stalling events, the generation of twist, whether the DNA strands are separated or not and the exact nature of the event that triggers DNA cleavage are still open questions. However, it is fair to say that, above all, what would really complement the work of Seidel *et al.*[9] and advance our understanding of these machines is a molecular structure, even if it is only at low resolution.

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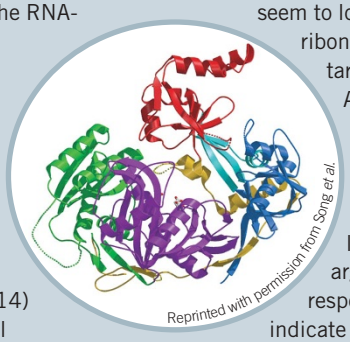
Finding 'slicer'

In most eukaryotes, double-stranded RNAs (dsRNAs) can trigger sequence-specific gene silencing. These RNA molecules are processed into short duplex RNAs ~21–25 nucleotides in length with 3′ overhangs of two bases. One strand of these duplexes is incorporated into a nucleoprotein complex called the RNA-induced silencing complex (RISC). The argonaute protein (Ago) recognizes the 3′ overhang of the single-stranded RNA (ssRNA) and is a crucial component of RISC. The loaded ssRNA guides the search for mRNA with complementary sequences and defines the site of cleavage (~10 bases from the 3′ overhang), but the identifity of the RNA cleaving activity ('slicer') in RISC was not known.



A recent *Science* paper (Song *et al.* published online 29 July 2004 doi:/10.1126/science.1102514) reports the crystal structure of a full-length archaeal argonaute protein. The structure reveals that the conserved PIWI domain (purple) has a RNase H fold with an intact 'DDE' motif (ball-and-stick) important for cleavage by RNase H. The PIWI domain forms part of the crescent base, and the PAZ domain (red), which has been shown to recognize the 3′ overhang, hangs above the PIWI domain. The structure suggests that argonaute may function as the slicer and the authors model in the ssRNA as well as the mRNA.

In mammalian cells, four argonaute paralogs have been identified, but it was not clear whether each protein could affect gene silencing. Two independent studies by Meister *et al.* (*Mol. Cell* **15**, 185–197; 2004) and by Liu *et al.* (*Science*, published online 29 July 2004 doi:/10.1126/science.1102513) have now characterized the properties of the human argonaute proteins. Both studies show that the human argonaute proteins seem to load ssRNAs indiscriminantly, but only the ribonucleoprotein complex containing Ago2 can cleave target mRNAs. Liu *et al.* also show that mice lacking Ago2 die as embryos because they have severe developmental abnormalities. These results suggest that the assembly of the catalytic Ago2 complex may be required during early mouse development. Furthermore, mouse embryonic fibroblast cells lacking Ago2 but expressing the other three argonaute proteins cannot silence gene expression in response to a small interfering RNA. These observations indicate that Ago2 has a specialized biological function distinct from the other paralogs. Finally, Liu *et al.* mutated two of three residues in human Ago2 that correspond to the DDE motif in the archaeal argonaute protein; these mutations abolish the RNA-cleaving activity of the human Ago2-containing complex. Taken together, these studies pinpoint Ago2 as the slicer in the RISC complex. Why do the other argonaute proteins, which also have the conserved PIWI domain, lack the RNA cleaving activity? Is each of the argonaute proteins involved in a specific pathway of dsRNA-mediated gene silencing? Future studies will have to answer these interesting questions.

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